Florida Huanglongbing Science Panel Report

Ben Hill Griffin Auditorium, Rm #1
Citrus Research and Education Center
700 Experiment Station Rd
Lake Alfred, FL

Date: Tuesday, 31 January 2006

The mission of the Florida Huanglongbing Science Panel (SP) is to provide guidance to state and federal officials for a huanglongbing (HLB) response that is based on sound science. Towards that end, scientists met on 31 January, 2006, to provide scientific recommendations on a number of topics. Those in attendance are listed in Appendix I. The meeting was organized by Wayne Dixon, Phil Berger, and Tim Gottwald (see agenda in Appendix I).

Opening comments

David Kaplan stated that we are in need of the best scientifically-based information to best understand what the regulatory response should be to huanglongbing. Based on this information there is a need to move forward with an appropriate response program. The guidance from this science panel will be important to the future development of a regulatory program. Wayne Dixon noted there was a key meeting on Monday (30 January 2006) to further develop the Citrus Health Response Plan (CHRP). Key elements of CHRP have been identified (e.g., nursery and budwood, production practices, packing and processing, harvesting and residential citrus) that will identify minimum regulatory standards for citrus production. It is imperative that standards specific to HLB be integrated into CHRP.

Science Panel Report

The SP was provided a series of topics covering regulatory, control, diagnostic, and survey issues to discuss. The SP addressed high priority issues, and will attempt to address remaining issues at later meetings or conference calls. To put this in perspective, Tim Gast, citrus horticulturist for Southern Gardens Citrus, US Sugar, described the HLB situation in a large commercial grove. Several areas of high disease density with edge effects have been observed. Currently, there are ca. 800 field positive trees out of one million trees. It appears that the disease has been in the grove for at least two or more years. A visual survey for HLB-related symptoms suggests that infection may range from 2 to 34% in some blocks.

The Select Agent (SA) Status of HLB

Because *Candidatus* Liberibacter asiaticus and *Candidatus* L. africanus are select agents, there are additional regulatory challenges and limitations on working with the microbes. The SP addressed two key concerns:

I. There is a desire from the diagnostic community to be able to possess infected tissue (e.g., dried, frozen or lyophilized tissue) so that comparable positive controls are available for diagnostic tests on an as–needed basis. These diagnostic positive controls are essential to achieve and maintain quality and reliability of laboratory-based diagnostics. Current rules state that although a diagnostic lab can accept HLB-suspect samples for testing, any sample proving HLB-positive must be destroyed no less than seven days after testing. To address this, the SP considered whether the pathogen can be recovered from these positive control samples.

    i. There are fundamental characteristics of the HLB pathogen that would indicate that there is little or no risk in allowing diagnostic labs to possess infected tissue that has been processed:
        - The HLB select agent is a fastidious microorganism that has never been cultured.
        - The principle means of pathogen transmission is by an insect vector or by grafting.
        - While theoretically possible to recover the pathogen from processed tissue, this is a highly technical procedure and for which such a recovery has never been demonstrated (see below).

    ii. There are two reports from China indicating that the pathogen was sap-transmitted from citrus to *Cantharantus roseus* (periwinkle) (Li, T. and Ke, C., 2002. Acta Phytophylacica Sinica 29:31-35; Fang et al., 2004. Acta Horticulturae Sinica 31:803-806). While these data suggest that *Liberibacter* spp. might be sap-transmissible, it is important to note that periwinkle is a transmission is accomplished using dodder. The SP was not aware of any reports of mechanical transmission to citrus.

II. Could HLB be used for Bioterrorism purposes?

    a. The panel was asked to consider in a more general sense the status of *Liberibacter* spp. as Select Agents (SA). The SP compared *Liberibacter* spp. to plum pox virus (PPV) because: the two pathosystems are similar; both agents are present in the US and are of relatively limited geographic distribution; both affect woody perennials; and both are vector-borne and graft-transmissible. PPV was once a SA, but has subsequently been removed from the SA list.

ISSUE	PPV	HLB bacterium
• Possibility of the organism to be used as weapon	Low	Low
• Lab research in the absence of its natural vector and only known means of transmission; how easily can it be obtained.	Poses little to no risk to plant health or plant products. Graft and sap-transmissible. Only known to occur in very limited locations in PA – would be difficult to locate in US.	Poses little to no risk to plant health or plant products. Graft-transmissible, but probably not sap-transmissible. It is readily and more easily available from the environment (in S. Florida).
• Transmission mechanisms	Sap-transmissible, and transmission by multiple spp. of native aphids	Probably not sap-transmissible; only by psyllid vector (2 species known, one in US)
• The natural host range is limited largely to plants in one genus	Primarily *Prunus* spp.	Mostly in the *Citrus* genus and some other Rutaceae
• The natural spread of the disease requires insect vectors, and is a complex biological process; artificial spread requires grafting, which is labor intensive, technically demanding and time consuming	Yes. Transmitted naturally by aphids. Intentional aphid transmission (i.e., acquisition and subsequent transmission) Relatively easy to accomplish with suitable source and host material. Graft-transmissible.	Yes. Transmitted naturally by two psyllid species. Intentional psyllid transmission more difficult. Graft-transmissible.
• Spread by pollen or seed.	Not known to occur.	Not known to occur.; under investigation
• Are systemic treatments effective at mitigating the disease	No.	Psyllid control may reduce HLB spread, but not yet published in a refereed paper.
• Destruction of infected trees mitigates the effects of the disease. Removal of the diseased trees and other susceptible hosts removes the source of infection	Yes.	No scientific documentation that tree removal will adequately control HLB.

Considering the ease with which HLB-infected budwood could be obtained from the South Florida environment, the Select Agent status of the HLB pathogen seems unnecessary. The SP does not believe that *Liberibacter* spp. pose a significant risk in terms of use as agents of bioterrorism.

Biological Control

The SP considered the usefulness of biological control of the vector to reduce spread of HLB. Biological control was considered in the context of the entire program, as well as a biological control proposal/scoping document submitted by Dale Meyerdirk (PPQ). The proposal recommended the development of the PPQ facility in Mission, TX as a location that could provide support of rearing of psyllids and parasites, and efforts aimed at foreign exploration.

While the SP recognizes the inherent value of biological control of insect pests, the value of biological control in this specific pathosystem is quite limited, and may not be a wise use of limited resources: (The SP is aware of current BC efforts in Florida.)

- While biological control and vector reduction in general appears to be a good concept, Asian citrus psyllids have a high reproductive rate which makes effective arthropod biological control a challenge.
- There is no example that the SP was aware of where biological control had an appreciable effect on a vector-borne plant disease. (One possible exception is the HLB infestation on Reunion; however, this is special case since Reunion is an island and therefore a closed ecosystem.) It should also be noted that results of an email questionnaire that was distributed to world experts, as reported to the SP by Dr. Susan Halbert, indicated that all who responded to this question (concerning the potential effectiveness of biological control for HLB) were unaware of any example where psyllid biological control had ever provided economically significant control of an HLB epidemic. (Michaud, 2004. Biological Control 29:260-269; 2002. Entomol. News 113:216-222; 2001. Florida Entomol. 84:608-612.)
- Although there was discussion on the preference for biological control in residential, organic farms and environmentally sensitive areas, the SP had doubts as to whether biological control would significantly slow the spread of disease in these areas.
- In the event the Asian citrus psyllid is observed in the Western States, pursuit of biological control research and development should be reviewed relative to these infestations if the psyllid infestation is determined to be of very limited distribution.
- In general, there may be merit to including cooperators within the research community, leveraging other resources, or finding external sources of funding which would help facilitate future discovery and development of biological control.
- There was consensus that some research effort be put into investigating entomopathogenic fungi which may prove useful as biopesticides.

The overall conclusion of the SP is that the probability of controlling HLB using biological control is low.

Insecticidal control is likely to be more effective than biological control in reducing psyllid populations and reducing disease spread. Control of psyllids and other insects in commercial groves is likely to be addressed only through the use of insecticides, and thus would not be compatible with a biological control strategy. The SP agreed that control funding, especially from a regulatory standpoint, would be better spent on other, higher priority efforts.

Is removal of trees positive for HLB necessary to stop the disease spread?

The SP discussed tree removal in terms of what to do with trees that test positive for HLB. Currently, the Florida Department of Agriculture and Consumer Services does not mandate removal of HLB-positive trees. Tree owners are encouraged to remove these trees if possible. The removal of diseased trees is viewed in the context of biological and regulatory perspectives: the effect on an epidemic when inoculum is removed or reduced versus the regulatory impact of removing a tree. Although removal of an infected tree will reduce the inoculum available for dispersal, the impact depends, in part, on the overall levels of disease and psyllid vectors in the general area. In other words, if the disease achieves a high incidence, tree removal will not be as effective. From a regulatory perspective, any infected tree in a production system should be quickly rouged, as the producer cannot afford to allow fruit from these trees to be harvested. This is because infected fruit may have an altered undesirable flavor that will potentially taint extracted juice flavor if the proportion of affected fruit is too high. Additionally, infected plants serve as sources for infective psyllids that pose a threat to the remainder of the grove.

Trees that have been exposed to infectious vectors presents a different problem, and recommendations regarding these trees will vary with the situation. At present there is no way to predict if exposed trees are likely to have become infected, nor when they may show symptoms of the disease (see also Diagnostics, below).

Does the SP's consensus of no feasibility of HLB eradication in Florida reached during the November 5, 2005 SP conference call still apply in light of what is known today?

In light of the known current distribution of HLB in 12 counties in FL, the SP reconfirmed that HLB eradication is not feasible. Future scientific emphasis should be directed to meeting the needs of regulatory and educational stakeholders. Key issues identified include whether Murraya (see below) is a true host plant of the pathogens. The scope, extent and benefit of disease quarantine and tree removal needs further discussion. Public outreach certainly deserves further attention as the disease is better understood and management strategies are developed for principal areas of activity (grove production, fruit harvesting, nursery and budwood operations, and residential properties).

Is quarantine in infected areas still necessary?

The SP agreed that commercial nursery stock production should be conducted away from areas of citrus production. A nursery should be quarantined if HLB is found within one mile of the nursery property. In a commercial grove, the SP concluded that a grower will make HLB management decisions based on fruit quality and tree health. Considering the general observations made regarding the disease and the vector: i.e., HLB can be detected in fruit peduncles and psyllids can move on unprocessed fruit, it was agreed that area wide management is important (such as proposed in the CHRP). Currently, in Florida, HLB hosts are prohibited from movement. Host plants of the Asian citrus psyllid are allowed to move if no psyllids are present—i.e., apparently psyllid-free. Some early testing of psyllids collected from *Murraya paniculata* at ornamental nurseries has resulted in at least four HLB- positive psyllids out of 250-1000 psyllids tested. The Division of Plant Industry, Advanced Diagnostics Laboratory has obtained a positive nested PCR for HLB in *Murraya paniculata*; however, a PCR "positive", *i.e*. the presence of *Liberibacter* DNA, does not imply that viable bacteria are present. Further testing and electron microscopy confirmation of *Murraya* infection is ongoing.

Is *Murraya paniculata* a host?

This is an important question, considering it is a host of the psyllid and is a common ornamental plant. Dai et al. (Dai et al. 2005. Research Bull. Plant Protection. Japan 41:53-57; in Japanese), studying the host range of *L. asiaticus* indicated that *M. paniculata* was not susceptible. For their study, they grafted HLB-positive citrus scions onto *Murraya* plants. Subsequently, PCR testing showed no grafted *Murraya* were positive for HLB.

However, recent reports from Brazil indicate that *Murraya* can serve as a host for L. americanus. The SP recommended that this work needs to be repeated with domestic varieties of *M. paniculata* and the Florida isolate of HLB. Dr. Vern Damsteegt indicated that work is underway at Ft. Detrick to experimentally attempt to infect *Murraya* sp. with HLB.

Some early testing of psyllids collected from *Murraya paniculata* at ornamental nurseries has resulted in at least four HLB- positive psyllids out of 250-1000 psyllids tested. It is not certain whether the psyllids acquired the bacteria from the M. paniculata or from other HLB host plants. The Division of Plant Industry, Advanced Diagnostics Laboratory has obtained a positive nested PCR for HLB in *Murraya paniculata*; however, a PCR "positive", *i.e*. the presence of *Liberibacter* DNA, does not imply that viable bacteria are present. Further testing and electron microscopy confirmation of *Murraya* infection is ongoing.

Is there a possibility of seedborne transmission?

There may be no connection between developing seed and the parent plant's vascular system, precluding introduction of the pathogen into seed. However, we do wonder why do seeds abort? Is there perhaps a mobile component produced by the bacteria? These are areas in need of research. Some work is underway in a collaborative effort between Drs. Halbert and Hartung to try to determine if seed-borne transmission of HLB occurs.

What is known about the pathogen/vector relationship?

The pathogen is thought to be multiplicative in the vector. There are definitive experiments that could be done, but these are technically demanding and time-consuming. Clearly, since transmission can occur over the lifetime of an insect, or nearly so, the issue of multiplication in the vector, while of scientific interest, is not particularly relevant in terms of the immediate needs of the program. The issue of whether the pathogen is transmitted transovarially seems more important, considering the risk of inadvertently moving potentially infected psyllid eggs. There are conflicting reports as to whether Liberibacter spp. are transmitted transovarially (Buitendag and von Broembsen, 1993. Proceedings from the 12th Conference of the IOCV 269-273; Roistacher, 1991. *A handbook for detection and diagnosis of citrus and virus-like diseases.* pp. 35-46; van den Berg et al., 1992. Israel Journal of Entomology 25-26:51-56; Xu et al., 1988. Proceedings of the 10th Conference of the International Organization of Citrus Virologists 243-248). Xu et al. (1988) reported that there is no evidence for transovarial transmission, because *D. citri* nymphs collected immediately after hatching on diseased plants did not transmit HLB to indicator plants. It is important to note that the most extensive studies on transovarial transmission of HLB pathogens were done with *T. erytreae*.

van den Berg et al. (1992) allowed immature psyllids to develop on heavily infected plants. When adults emerged, they were allowed to feed and mate on infected plants. After 14 days, the mouthparts of 100 of the females were severed. Ten of these females were placed on each of ten healthy indicator plants, where they laid eggs. Adults from those eggs were allowed to feed on the same plants for 30 days after emergence. Plants were later sprayed, kept insect-free, and tested for HLB after six months. One of the ten plants developed HLB. In another experiment, oviposition was allowed to occur on the infected plants. Crawlers were removed immediately after hatching and prior to feeding and placed on indicator plants. Five of the 24 plants on which these psyllids completed development became infected with HLB. The most logical explanation for these infections is transovarial transmission. However, the authors postulate that the plant in the first experiment could have been infected via oviposition, and those in the second experiment could have been infected as a result of absorption of HLB bacteria from the infected host by the egg. The SP recommended that these experiments should be repeated with *D. citri* . Dr. Vern Damsteegt indicated that research along these lines is being considered by the ARS in Ft. Detrick.

How far might HLB be dispersed by psyllids?

This is an important question, particularly relative to CHRP and the safety of the Florida citrus nursery industry. The SP was asked if greater than one mile separation between an HLB-infected citrus tree and screened nursery stock is adequate? The short answer is probably not. There is some information indicating that *Trioza* may fly 1-2 km (0.6 – 1.2 mi) and suggestions that the Asian citrus psyllid may do the same. Long distance dispersal has not been documented.

- Circumstantial evidence from observations in Florida suggests dispersal of infectious psyllids of up to 50 miles.
- Work from Brazil indicates HLB dispersal by both insects and movement of infected plants is between 12-15 miles per year.
- There is considerable speculation or circumstantial information on the frequency and distance of psyllid dispersal.
    - In a non-refereed report Aubert (1987) states: "...recent observations suggest possible medium to long transports by strong winds, since upsurges of *D. citri* have appeared in open orchards without windbreak protection, after typhoons (especially Northern Philippines and South East China coast). Passive transport through advective winds seem much more occasional." (Aubert, B, 1987. Proceedings of the Regional Workshop on Citrus Greening Huanglungbin Disease, Fuzhou, China 5pp.)
    - Y. Sakamaki (2005) speculated that the Asian citrus psyllid could move between the Japanese islands of Yoron and Kuyushu, a distance of 270 miles. (Y. Sakamaki, 2005. *Occasional Papers of the Kagoshima University Research Center* 42:121-125.) While admittedly speculative, it is based on the following (paraphrased from Sakamaki's paper):
        - Density- it is well-documented that insects disperse when they surpass peak densities. Peak density for the psyllid has been calculated (by Sakamaki) at 1 adult per new shoot since each female lays about 626 eggs (Tsai and Liu, 2000. Journal of Economic Entomology 93:1721-1725). Any higher than that and they should disperse. From studies by Hayashikawa and Torigoe (2004) on Amami Island, peaks were often reached numerous times a year, leading to many opportune times for dispersal events.
        - Wing structure- The flying muscles of a strong flyer, the vegetable leafminer, was compared with the Asian citrus psyllid. The surface area of the flight muscles is much smaller for the psyllid than the dipteran. Also, the wings of the psyllid are much larger (and both are used) and have a relatively low stroke frequency, like

the brown plant hopper, suggesting that the psyllid passively soars in wind currents much like a kite. Thus, the citrus psyllid is very similar to the brown plant hopper in this respect. They are both basically the same size, and are weak fliers. There is considerable evidence demonstrating the brown plant hopper's ability to migrate large distances (Mills et al., 1996. Bulletin of Entomological Research 86:683-694), and so it is reasonable to believe that the psyllid could similarly disperse over great distances.

    - Psyllid migration within Yoron Island follows seasonal wind patterns and moves from south or west, to north or east during June to August, and moves in the completely opposite direction when the wind changes in February to March, and October. (How this was determined was not stated.)
    - Jet stream (geostrophic) winds. If the psyllid population density is high, psyllid adults may disperse. They could be taken up into the jet stream which can carry them from Yoron to Kyushu which is about 270 miles away in approximately 17 hours. The psyllid was probably introduced to Yoron Island by human activity, but may spread between islands through wind dispersal.
  - It is worth noting that other psyllids (not ACP) have been observed at altitudes of 150 to 7,550 ft.
  - By analogy, droplets of water with citrus canker bacteria have been known to disperse up to 35 miles and cause infection.
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It is the opinion of the SP that nursery stock produced within any proximity to possible populations of infectious vectors are at risk. However, there is no scientific information to allow the SP to make recommendations on a 'safe' distance. There is no guarantee that greenhouses or screen houses will provide complete protection from psyllid incursion. In general, an insect-proof structure is next to impossible to achieve. Stock for budwood, which is being moved to north FL, is especially at risk. At present, the nursery industry has expressed it has little confidence in being able to produce HLB disease-free stock. However, the SP recognizes there is a need to assist in the development of regulatory options to achieve the production of certified nursery stock.

Clearly, there is a need for more information on disease epidemiology and vector biology.

What is the drop dead date for nurseries in FL with open air trees to stop selling?

Our current understanding of disease spread and difficulty of detecting presence or absence of the disease with certainty suggests that field grown nursery stock should be viewed as high risk for disease spread into uninfested areas.

What chemical control strategies should be used in commercial groves?

UF/IFAS is currently in the process of developing recommendations on what products could be used for psyllid control. There is one soil-applied systemic insecticide, aldicarb (Temik), available and a number of other chemical controls that may be applied to mature trees. However, it is unknown if there is a direct link between insecticide use and reduction of the spread of HLB. It was noted that imidacloprid (Admire) is approved for use on small trees and containerized material. The same material used on large trees exhibits translaminar flow and needs to be sprayed at 14-day intervals. The SP agreed that abandoned/unmanaged citrus groves present specific problems and will need regulatory attention. It is critical that feral citrus in Florida be eradicated as soon as possible. This is not only important for the management of HLB in Florida, but also for other citrus pests and pathogens including canker, Medfly, and Mexfly. The SP also learned that research is underway at the UF Citrus Research and Education Center on the effects of imidacloprid on psyllids. Similarly, Dr. Vern Damsteegt is conducting an experiment to determine whether infective psyllids can transmit HLB to citrus plants protected by imidacloprid. The SP agreed that abandoned/unmanaged citrus groves present specific problems and will need regulatory attention. Psyllid control may need to be truly area-wide in order to have an effect on disease incidence.

What is the usefulness of sampling psyllids for survey purposes?

It has been suggested that collecting psyllids and assaying them for the presence of the pathogen may be a useful survey tool, particularly in areas where the vector is present, but the disease has not yet been observed. Psyllid sample compositing was discussed; however, the SP is unaware of any data on this technique. It may be an unanswerable question, as a properly controlled experiment would be technically difficult, and there is no way to control the amount of bacteria obtained by individual vectors.

While this seems, at first glance, a good way to at least determine that the pathogen might be present in a particular area, it would likely provide limited epidemiological information. Prior work has indicated that the proportion of infected psyllids in an area is in direct proportion to the incidence of HLB-positive trees in the area. The proportion of psyllids that are sampled relative to the proportion of citrus trees that will be examined is enormously different. At best, even if insects were sampled in groups of perhaps 100 (there is no data to prove that one can reliably detect one infected individual out of 100), in terms of a National Survey (and in Texas, where the vector is known to occur) only an infinitesimally small proportion of the psyllid population will be sampled. Thus, the SP does not recommend that psyllids be surveyed at this time as a survey tool in Texas. It was felt that the system that is currently being used in Florida would be effective elsewhere, whereby samples that are considered to be highly suspect, on the basis of symptoms, receive priority for diagnostic testing, samples that are medium are next in priority, and so on. Psyllid samples should receive the lowest priority. Also, preliminary research did not discern relationship between highly HLB-positive psyllids and citrus plant expression of disease after psyllid feeding.

There was some discussion of the potential for trapping of psyllids for survey purposes. Yellow sticky traps were observed to be inefficient for psyllid detection. At present, a psyllid-specific pheromone is unknown and it was speculated that the insect may not produce such an attractant. Research is needed to better answer this area of concern.

(Note that the SP was provided estimates of funds available for the upcoming National Survey and limitations that that funding imposes on the number of samples that can be tested.)

What are the current survey activities in Florida?

There is a need from the regulatory standpoint to determine the distribution of HLB in the southern peninsula of Florida. Currently, TDY USDA and DPI contributions have 45 people in the field, and there is another week or so left in this rotation. DPI will continue intensive surveys around nursery sites over the next several months. The question posed to the SP is: should there be continued TDY or intensive survey?

The SP noted that although latency of HLB in a citrus plant means surveys are 1-3 years behind the actual time of disease infectivity, there is still a need to determine disease presence, prevalence, and distribution. Currently, there is a fruit fly trapping network in Florida that could provide an additional opportunity to inspect trees for HLB symptoms. Specific citrus canker/HLB surveys by regulatory agencies are worthy endeavors because citrus canker will likely continue to be a regulated pathogen and therefore surveys should be a part of the developing CHRP. The SP agreed that grove self-inspection surveys would be important contributions to the overall detection of these diseases.

Should efforts be made to develop surveys in packinghouses?

Yes, especially for Texas, Arizona and California. Large portions of the fresh fruit crop pass through packing houses and thus packing houses are good central locations to monitor for symptomatic fruit. Survey resources will need to be identified and in place at the packinghouse to be able to accomplish detection and achieve any trace back to a commercial grove.

What are the diagnostics issues?

- Diagnostics for this pathogen are reasonably robust and sensitive. However, the pathogen is presently patchy in distribution in Florida and may be present at low titer in some citrus samples. While diagnostics are robust for the strain known to occur in Florida, it is unknown how existing assays would perform if new pathogenic *Liberibacter* spp. or pathotypes were present in the US. Primers generic for *Liberibacter* r16S have been developed by the ADL, DPI, that are based upon the presently known species and, in fact, were used for the initial identification of *L. asiaticus* in Florida; however, it is impossible to predict their performance on currently unknown taxa. There needs to be continuous vigilance in looking for different pathotypes of the pathogen.
- Ensuring that as many isolates as possible are collected for maintenance at Beltsville is important, since there is a need to be able to further examine these isolates to determine if different pathotypes are present and to permit investigations into genotypic diversity.
- Research is needed to determine if it is possible to detect an asymptomatic tree. For example, studies on citrus gene expression, facilitated by the use of microarray technology, might identify genetic elements that respond to HLB long before symptoms appear, thus affording the possibility of detecting these plant responses before symptom expression. Applied results of this work could be three or more years away.
- It would be useful to develop antibodies for use in immunoassays, but this is not likely to occur in the foreseeable future.
- As more causal agent genetic information is obtained, the likelihood of improved diagnostic tests increases. Efforts are underway to attempt to sequence the genome of the Florida HLB isolate.
- When is a negative a negative? It is imperative that a proper error analysis of the protocols for the detection of *Liberibacter* in citrus is carried out. As it stands it is not possible to "detect" *Liberibacter* in citrus in any meaningful sense, only to verify the taxonomic the identity of the pathogen. For a given sensitivity threshold, defined by a certified standard, what is the probability that a biologically meaningful titer of pathogen is present for a "negative" defined within the context of that threshold?
- What should be done when samples with strong symptoms result in negative test results?

It is to be expected that bacterial pathogen die-off will occur with the build up of metabolites and decomposition products from both the *Liberibacter* as well as affected citrus tissue. This situation is currently under investigation by the ADL, DPI. The SP recommends in situations as indicated above, that all tests/assays available be used on the sample. Any sample that has strong symptoms yet produces negative results or results that are ambiguous should be sent immediately to Drs. John Hartung and Yongping Duan for further study.

Literature

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Florida Huanglongbing Science Panel

Ben Hill Griffin Auditorium, Rm #1

Appendix I

Citrus Research and Education Center
700 Experiment Station Rd
Lake Alfred, FL

Date: Tuesday, 31 January 2006
Time: 8:45 AM – 5:00 PM

Mission: To provide guidance to state and federal officials for a huanglongbing response that is based on sound science

Expectations for the Day's Work: Report on recommendations and action items

Introductions
Science Panel Membership
Organization – Chairman(s), Subcommittees
General Session
Subcommittee Breakouts: no later than 10:00 AM
Lunch
Presentation and Review of Subcommittee Recommendations and Action Items
Report Assignments
Adjourn

Science Issues

The following is not an all-inclusive list, more it is to show the complex science challenge of the huanglongbing, vector, and host complex.

Diagnostics
- Improvements/advances in disease diagnostics (field and laboratory)

Detection of disease low titer
Certainty of disease and strain diagnostics
Rapid diagnostics in field and laboratory
When is a negative a negative?
- Strategy to detect new strains of HLB at incipient field levels
    - Molecular biology
        Sequencing, culturing, genetics and genomics

Transgenics

Survey
- Survey methodologies for effective and efficient detection and delimitation of HLB in multiple hosts and environmental/host settings (commercial grove, nursery, retail outlets, residential, Asian farms)

Appendix I

Host plants with and without symptoms
Psyllids – trapping, pheromones, baits
Nymphal populations vs. adult populations

- Seasonal phenology of disease expression

- Data needs for short-term and long-term analyses of disease epidemiology and vector movement
- Inspection frequency

Control

- Strategies for removal of positive and nearby (exposed, latent, etc.) trees
- Best management practices (BMP's) for groves and nurseries where HLB occurs and for where it is not known to occur
- Chemical controls and strategies for adult and nymphal psyllids on (large) trees in groves, nurseries and residential trees
- Biological control of psyllids in different host settings: grove, nursery, residential
- Foreign exploration for biocontrol agents

Regulatory

- Testing of potential host plants: *Murraya paniculata et al.*
- Movement of plants such as *Murraya*'s and Calamondins
- Development and maintenance of host plant list
- Host plants: discernible field symptoms vs. initial colonization
- Insecticides for phytosanitary permits: efficacy and residual toxicity relative to plant shipments
- Labeled product availability for groves, nurseries, retail and residential
- Quarantine strategies in areas of diagnosed HLB
- Movement of fruit: seed transmission, psyllids
- Determination of whether bacteria multiply in psyllids and occurrence of transovarial transmission
- Performance of Koch's Postulates

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Appendix I

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HLB Science Advisory Panel Questions from the Program
Provided April 14, 2006

Survey

1. When is sampling psyllids via PCR analysis appropriate / useful in the survey program? Can composite samples of psyllids be made from Texas collections and a PCR diagnostic be applied for surveying larger growing areas?

This is covered in the SP report. The basic answer is no. It is not likely to be time or cost effective to sample psyllids for the purpose of survey. The pathogens remain latent inside the vector from between 3 to 20 days, after which they can be detected in the salivary gland (i.e., if the pathogen replicates in the vector, then detection will change depending on the time after acquisition). The percentage of "hot" psyllids in a population is highly variable and poorly known. For example, Teixeira et al. (2005) reported that only 6 of 22 samples of 10 psyllids each from 3 HLB infected plants with severe symptoms were PCR positive.

2. Assess the relative effectiveness of each of the general detection survey strategies? Which strategy is best under what conditions? Which strategies provide the most information? Which ones are more cost-effective? (Effectiveness= defined as most likely method of detecting HLB in various settings [residential, nursery, groves, retail outlets, etc.]; if a numerical expression of effectiveness is provided, the panel is to also provide a cost figure attached to it).
    a. Hot-zone/demographic
        i. Useful to target survey efforts toward detection of new foci of infection of exotic pathogens when very limited in distribution and there is a suspicion that a potential pathway from known foreign areas potentially exists. Program would benefit from effective outreach and training programs.
    b. Sentinel residential survey
        i. Useful to detect relatively low incidence infections and foci in residential areas. Will also detect more extensive infections.
    c. Sentinel grove survey
        i. Useful to detect low incidence infections in commercial plantings. Can be used to detect more extensive infections as well.
    d. Current rapid delimiting HLB survey using transects/concentric annuli
        i. Useful to target survey efforts toward detection and delimiting of new foci of infection of exotic pathogens. When very limited in distribution, this method will rapidly delimit the infection. If infestation is extensive, rapidly helps to find the proximal edge of the epidemic wave.
    e. Vector survey

i. Limited usefulness because the total population of vectors is very large and the number of infected vectors is directly proportional to the number of infected hosts in an area. Thus only when the proportion of infected hosts is high will infected vectors prove a useful tool for detection. When infected host density is very low or low, the probable success of detecting the presence of disease via sampling for infected vectors is very unlikely. Since how the vector disperses is poorly understood, presence or absence of HLB+ vectors may not be of use to determine host infection in an area.

f. Self inspection by industry and home owners

i. Very useful when disease distribution and incidence is still relatively unknown and it is important to determine distribution and areas free of disease. Engages a very large number of dedicated and interested people directly in the detection at virtually no regulatory cost except training and available public information.

3. After confirmation in a commercial grove, what is the methodology for a delimiting survey in that grove? It is to determine, i.e. with large groves, where control actions may be necessary within a very short period of time. For instance, the "delimiting survey" is to be conducted/completed within one day.

This would depend upon the detection/confirmation method used. For example, is visual detection sufficient or do we need to detect asymptomatic infections as well? In either case you need a stratified survey method that can be applied at two different spatial scales. The stratified method will allow detection at some predetermined threshold level of incidence and not necessitate examining every tree. A hierarchical survey (HS) would be preferred as the statistical appropriateness has been demonstrated for grove survey. If the grove is relatively small, each block can be surveyed via HS. This would be an intensive survey of all blocks. If the grove is very large, then blocks to be surveyed can be stratified across the grove and each surveyed via HS. This will allow for a quick sweep of the entire grove and give a general estimate of incidence in all areas of the grove but it will be incomplete and will miss some infections. All this said, one could argue for a 100% survey since no pre-determined distance is available for tree removal (if this is the action of choice).

Survey methods within a tree will need to be determined. Brazil and Argentina use aerial platforms to look at the tops of trees which is where greening symptoms are more frequently found first.

There would need to be recommendations/guidelines for a survey based on a positive detection of a reset in order to determine if the resets were infected originally and to identify and locate these in the grove.

4. Are there survey data not currently being collected that could assist in long-term evaluation of the problem?

We definitely need a statewide comprehensive survey to establish the distribution of HLB in Florida and to establish the approximate incidence of HLB in various areas where it has been detected. It is nearly impossible to recommend regulatory measures without this information.

It was stated at a Citrus Health Response Plan meeting that FL would be conducting intensive surveys around nurseries.

5. Is there research that indicating a possible pheromone for Asian citrus psyllids?

There are some reports (probably unpublished and therefore anecdotal) where attempts were made to find a pheromone. This work was apparently unsuccessful. Opinions in the SP were expressed that this work needs repeating, but it is possible that the ACP does not make use of a pheromone, or at least not in such a way that it could be useful to the program. There is no work that we are aware of identifying a pheromone for the Asian citrus psyllid. Hall 2006, Citrus and Veg. Magazine, stated “With respect to traps for adults, there is no attractant currently available for survey purposes.”

Diagnostics

1. Can more rapid field-deployed diagnostic tests be developed, such as a “dip stick” (serological) test and how long would that take?

There are no useful antibodies for this pathogen. Those that have been produced appear to be too specific for our purposes. “Monoclonal antibodies raised against proteins purified from infected greening tissue from Africa, China, and India reacted selectively with the source antigens and a few other isolates of citrus greening, demonstrating the existence of several serotypes of greening (Garnier et al. 1987; Gao et al. 1993). These monoclonal antibodies are too isolate-specific to be used for general detection of greening.” Halbert and Manjunath 2004, Fl. Ent. 87(3):330-353.Work is being initiated to attempt to produce antibodies, but this will take some time and there is no guarantee of success. The inability to culture the organism makes this quite challenging.

2. Are we absolutely certain that the strain of HLB we have in Florida is the Asian strain? Do we need to ensure we are not dealing with another citrus canker “nursery strain” situation?

There are few absolutes in biology. All data that have been obtained to date (PCR reactions, sequence of the amplicon where this has been done) are consistent with the identification of the isolate obtained in Florida as *L. asiaticus*. Thus, we have no information at this time to indicate more than a single ‘strain’ of HLB in Florida. Many of the collections have been archived in Beltsville, and in time we will have more information on potential differences between them. These samples will be eventually contained in the ARS Exotic Pathogens Collection. It was mentioned by the scientists from the Florida Department of Agriculture and Consumer Services at the 2nd Int. CC HLB workshop in FL that there was the possibility of other strains or variants in FL based on faint PCR or neg PCR with highly symptomatic tissue.

The presence of genetic variants was suggested as one possibility for “weak” or negative PCR for a subset of highly symptomatic tissue samples. All r16s and ITS sequences have been consistent with our present concept of (*Candidatus*) Liberibacter asiaticus. Cloning and sequencing ribosomal fragments generated from PCR negative but symptomatic tissue using r16s universal primers for bacteria has so far failed to suggest the presence of other Liberibacter.

3. What are the elements of the HLB proficiency test panel and laboratory approval program for local diagnostic testing?

PPQ CPHST is in the process of developing a proficiency test (PT) panel for this pathogen. Developing PTs for many plant pathogens is challenging, and HLB is no exception, particularly a test panel that would truly test proficiency with the diagnostic assay(s). Elements for the PT panel should include isolation of symptomatic tissue, DNA extraction, DNA amplification, correct diagnosis and interpretation.

In order to specify a formal regulatory diagnostic procedure for the detection of Liberibacter species it is critical that a certified performance standard be developed and the Type I and II errors determined with respect to that threshold. Proper validation of an assay should include obtaining this information, but applying these standards to a biological system may be more challenging that what is currently used for chemical and manufacturing systems. In addition, it is imperative that a formal sampling strategy for Liberibacter in citrus be developed based upon the distribution of this pathogen *in planta*, which also may prove to be rather challenging.

It should be noted that in citrus tissue exhibiting severe HLB symptoms PCR results are often negative. One reason for this may be that in this tissue the removal of PCR inhibiters by the presently used extraction methods is insufficient; however, there is no evidence supporting this scenario. It is may be that Liberibacter titer in the advanced stages of HLB drops, perhaps as the concentration of metabolites, plant decomposition products, etc. increase with the collapse of the plant vascular system. Another possibility is that that low titer in symptomatic tissue is a result of host defense responses.

Regulatory

1. Is *Murraya paniculata a* host of HLB?

Existing scientific literature indicates that it is not a host, based on work from Taiwan (Dai et al. 2005. Res. Bull. Plant Prot. Japan 41:53-57). This work will be repeated using domestic isolates of the pathogen and vector, at Ft. Detrick. There is evidence suggesting that *Murraya* may support HLB *Candidatus* L. americanus in Brazil. Halbert and Manjunath, 2004, indicate: “Careful work using dot hybridization by Hung et al. (2000) indicates that Asian greening pathogens from Taiwan will not multiply in *M. paniculata* or *M. koenigii*. Toorawa (1998), who worked in Mauritius, concurs. (On the other hand, Tirtawidjaja (1981) was able to observe consistent external and internal symptoms on 25-33% of inoculated *M. paniculata* plants. Asian greening may be caused by a population of bacterial strains with somewhat differing host ranges." However, symptoms alone are not suffient to conclude that the pathogen is present.)

PCR "positives" for (*Candidatus*) Liberibacter asiaticus from symptomatic *Murraya paniculata* leaf tissue from Florida have been obtained: however, the presence of HLB pathogen DNA is not sufficient to infer viable bacteria, particularly given the large numbers of "infected" psyllids present. The presence of symptoms in inoculated or field *M. paniculata* may represent plant response to injected non-viable and/or non-replicating Liberibacter. Bottom line: more work is needed.

2. What is the appropriate buffer distance for quarantine on host nursery stock near a positive detection of HLB? (a buffer of 4 square miles around a positive detection was just adopted in Florida).

There was considerable discussion on movement of the vector in the SP discussion. There is no single answer to this question, but the SP believes, based on existing information, that 4 sq. miles is probably not adequate, assuming that the nursery stock is produced under cover and protected as much as is possible from insects.

Sullivan and Zink (CPHST NWML), indicated – "The distance of dissemination depends on the population of the psyllas, wind velocity, and the survival time in the air. *D. citri* has been observed disseminating over a distance of more than 500 m in a flat field and *T. erytreae* has been observed disseminating over 1500 m. The length of survival in air currents is also unknown, but the possibility exists for hurricane force winds to aid in the dissemination of *D. citri* in Florida and Gulf Coast areas."

3. How long is the imidacloprid drench treatment effective for nursery stock? We need to determine a time frame whereby the treatment remains efficacious, at least from 10 days prior to shipment until the plants reach their final destination.

When applied to containerized plants, including citrus and other ornamentals, the current opinion is that imidacloprid is likely to have residual toxicity for three months and may be as long as six months. Imidacloprid is retained much longer in potting media which is high in organic matter and remains available for uptake by the plants over a longer period of time than say an application made to trees in the field with sandy soils where the imidacloprid does not bind well and can be washed away rather quickly. Research is ongoing within IFAS to better define the residual toxicity period of imidacloprid in containerized citrus. In discussions with Bayer CropSciences, they support that their product will provide at least 3 months control in containerized citrus. They also feel that control is actually much longer in containerized plants but do not have data to support that right now. So based on a lack of data to support a longer duration of control, containerized plants could be shipped if treated within the previous 3 months.

Bare root nursery trees cannot be protected from psyllids using soil applications of imidacloprid. Imidacloprid application to bare root trees does not work due to the high number of trees per acre and the low allowable use rate.

Ahmed et al., 2004, Int. J. Agri. Biol., 6:970-973 – imidacloprid produced up to 90% control of *D. citri* 7 days after application to 3 citrus species grown in the field.
Farmanullah et al., 2004, Songklankarin J. Sci Technol., 27:17-23
Thiamethoxam (Actara) systemic with knockdown effect, provided 72% and 84% decrease in psyllid populations per 6” shoot after 2 sprays 1 month apart.
It is currently not registered in FL for citrus or psyllids. It is more water soluble than Imidacloprid and a little quicker in action, but it does have a higher carcinogen concern than Imidacloprid. It should be registered in FL by 2007 or as late as 2008.

4. What is the research on additional alternatives for managing risk of citrus and ornamental psyllid host nursery stock in infected counties? What are the risks of not quarantining all movement of HLB and psyllid hosts from nurseries within a regulated / infested area?

The research is limited on additional alternatives. Two recent publications that are available for a broad description of current knowledge are:

Halbert, S.A. and L.M. Keremane. 2004. Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) And Greening Disease Of Citrus: A Literature Review and Assessment of Risk In Florida. *Florida Entomologist.* 87(3) September 2004.

da Graca, J.V. and L. Korsten. 2004. Citrus huanglongbing: review. present and future strategies. S.A.M.H. Naqvi (ed.). Diseases of fruits and vegetables. Vol. I, 229-245. Kluwer Academic Publishers. Netherlands.

As with any vectored disease, the lack or limited quarantine of HLB and psyllid hosts from nurseries within a regulated/infested area will not appreciably change disease spread.

5. How is a host of HLB to be defined? It will be useful to define a “host” with L. asiaticus. Is it when symptoms are expressed- perhaps 3-5 yr post infection (is infection defined as “colonization of the phloem of a plant”?).

Presumably, a ‘host’ of HLB is one which can be infected by either grafting or by inoculation by a psyllid, and further, from which the pathogen can be detected after an appropriate incubation time. There can be a distinction between a natural host and an experimental host, however. In general, it is difficult or rare to detect the pathogen in the absence of symptoms.

There has been some suggestion that HLB symptom expression may be influenced by plant nutritional status and health. Are there conditions under which Liberibacter may persist at low titer, perhaps with a plant exhibiting minimal or nil symptomology? It can be, at times, difficult to determine if what one detects after, say, psyllid inoculation, is due to replication of the organism or simply the original inoculum.

Control

1. If early detection were feasible in Texas, Arizona, and California, or in isolated areas of Florida, what would elements of a possible eradication/containment program include? If it is not feasible now, what are the obstacles to finding this information, and how can they be addressed in the short term? Is a more sensitive diagnostic tool the answer?

"Early detection" may be more an element of luck than by design, particularly if distribution and incidence are very low. Of course, as stated above, well educated and concerned growers is a key element. If information indicates that infection is limited or very isolated, eradication may be possible, but only under a relatively limited set of circumstances. It needs to be kept in mind that we are always one to three years behind the disease, making eradication very challenging. A more sensitive diagnostic tool is not necessarily the answer for early detection of the disease, due to its long latency and transmission via a vector.

2. Is there a reasonable distance for the removal of exposed trees to (1) minimize the disease and (2) perhaps eliminate it in an area? What has been applied in foreign countries to control HLB spread? How effective is it?

There is no single (or simple) answer to this question. See the SP report regarding dispersal/movement of the psyllid. Similarly, the SP commented on the likely effects of tree removal. While removing symptomatic trees *may* slow the spread of HLB, there is no way to know which exposed trees to remove. Furthermore, the asymptomatic trees probably do not serve as a significant reservoir for vector acquisition. In terms of commercial production, symptomatic trees would be removed in any case. It is likely that any citrus trees near an HLB-symptomatic tree are at high risk of HLB infection, but simply not expressing visible symptoms. In other parts of the world, they remove whole trees that are significantly infected and symptomatic parts of trees that are partially infected. In South Africa, Buitendag and von Broembsen (1993) worked out equations based on the age of the tree and the percentage of the tree infected (Buitendag and von Broembsen. 1993: Proc. 12th Conf. IOCV. 269-273) . Monitoring for disease symptoms is a regular part of most foreign programs. These tactics have been an important part of successful integrated citrus programs in HLB-endemic areas.

3. What are the best management practices (BMP's) for groves where HLB occurs and for groves where it is not known to occur? What has been applied in foreign countries to control HLB spread? How effective is it?

The common elements incorporated into most citriculture programs in countries dealing with HLB are 1) planting of disease-free certified nursery stock, 2) Regular applications of insecticides, timed with citrus flushes, in addition to monitoring of the psyllid vector, 3) removal of symptomatic plant tissue and/or HLB-infected trees. In addition to these basic management practices, some countries incorporate additional tactics. In Indonesia, for example, they eliminated all citrus from the area before replanting with disease-free stock (Bove et al. 2000. Proc. 14th Conf. IOCV. 200-206). In South Africa, they eradicate native plant hosts of HLB from the vicinity of orchards (van den Berg. 1999. Neltropika Bulletin. 203:5-6). In an experimental farm in China, in addition to all of the above, they have also eliminated all backyard citrus and hosts from the area and established strict quarantines, forbidding the entry of HLB hosts into the citrus growing area. All trees were supplied by a state-sponsored certified nursery. Pesticide applications averaged 10-13 times a year, only during flushes. Nine years after planting, there still was no sign of HLB, and the farm was located 2.4 km from a HLB-infected orchard. Other farms in China employing similar tactics have reduced HLB incidence to below 1%, and increased yields dramatically (Ke and Xu.1990. 4th Int. Asia Pacific Conf. on Citrus Rehabilitation. 145-148.; Roistacher. 1996. Proc. 13th Conf. IOCV. 279-285). HLB has been mostly eliminated in Réunion largely due to certified disease-free nursery stock, elimination of HLB-infected trees and biocontrol, which was successful in Réunion due to island biogeography and the lack of hyperparisitoids (Aubert et al. 1996. Proc. 13th Conf. IOCV 276-278). In Brazil, the simple version of HLB management consists of psyllid population control through chemical suppression, mandatory removal of HLB-symptomatic trees, production of disease-free nursery stock and a strong survey of commercial groves.

The BMPs for groves where HLB doesn't occur should be similar to those for groves where it does occur, with extra emphasis placed on quarantine; very strict controls placed on what is allowed into the area. Extra effort should be directed toward windbreaks, and elimination of HLB-hosts in a buffer zone around the citrus-growing area. (But, this does not necessarily address residential situations.) For Florida, the best management practices are still under development, but will likely contain similar elements to the Brazilian model. However, the mandatory removal of HLB-symptomatic trees is not currently required by FDACS, instead it is encouraged.

4. What are the BMP's for nurseries where HLB occurs and for nurseries where it is not known to occur? What has been applied in foreign countries to control HLB spread? How effective is it? What has been applied in foreign countries to control HLB spread? How effective is it?

In foreign countries that attempt to control HLB, nursery certification programs have been put in place and trees are generated in insect-free screen house usually in isolation. Some psyllid control is attempted in some locations usually with mixed or poor results.

It is certainly advisable to establish nurseries in HLB-free areas, but that hasn't always been an option for every program. Under these circumstances, extra precautions need to be considered. The following are components of successful foreign citrus nursery programs: 1) Areas around the production facilities need to be free of HLB host plants from at least 5 to 8 km. (Lin and Lin. 1990. 4th Int. Asia Pacific Conf. on Citrus Rehabilitation. 1-26). 2) Screen houses need to be built with psyllid-proof screens (Buitendag and von Broembsen. 1993: Proc. 12th Conf. IOCV. 269-273) and double door entries. 3) Quarantine programs prohibiting the entry of HLB hosts into the nursery zone. 4) Certification programs, with budwood from pathogen–tested, indexed mother trees, and sanitation programs for recovering important cultivars using shoot-tip grafting. The BMP's for nurseries in countries where HLB does not occur, such as Spain, are well-explained in Navarro 1993. Proc. 12th Conf. IOCV. 383-391. The main components of this paper are sanitation, quarantine and certification programs. These measures will ensure that the nursery stock is not only free of HLB, but free of canker, tristeza, and other pathogens.

(The only successful control of HLB in a foreign country has been through biological control, and that situation is only pertinent to island systems. Biocontrol and other attempts at HLB/psyllid eradication have not been successful on larger landmasses. The citrus industry has been producing economical yields in countries where HLB exists only through the implementation of stringent IPM programs as outlined above.)

5. Are there effective chemical controls for psyllids on large trees in groves? Are large tree treatments for psyllids less effective than on small trees- why? Is it a question of physiological or physical characteristics of plants of different ages that presents challenges for consistent psyllid control (management?)?

See, Browning et al., 2005 UFL IFAS pub. This publication discusses foliar insecticide applications during major flushing periods, and rotation of insecticides to avoid resistance in the psyllids.

Mike Rogers UFL, notes from the 2^{nd} Int. CC HLB workshop – Soil applications of imidacloprid applied to bearing trees greater than 8 feet in height are ineffective. The large trees have too much leaf mass and thus require more than a legal (and economically feasible) amount of material be applied to get adequate concentrations of imidacloprid translocated from the roots into the new flush. Aldicarb (Temik) is currently the only soil-applied systemic product that has been shown to suppress psyllid populations on large bearing citrus trees. Aldicarb is soil-incorporated and may only be applied from Jan 1 through April 30 in Florida citrus. With only one soil-applied systemic product available for use on large bearing citrus trees, foliar applied insecticides are the primary tool available for reducing psyllid populations in this situation, especially where there are restrictions on application of aldicarb. For foliar applications, chlorpyrifos (Lorsban), fenpropathrin (Danitol), and imidacloprid (Provado) are labeled for psyllid control and have been proven to be effective in IFAS trials. All three of these products are effective on both large and small trees and overuse of these foliar products has the potential to cause outbreaks of nontarget pests due to mortality of established natural enemies. New pesticides with novel modes of actions are being evaluated and we will likely have more recommendations after the 2006 psyllid field trials are completed.

Penetration of spray applications into the tree canopy is not as much of a concern because the area to be protected is the outer canopy of the tree where most of the new flush occurs. The obstacle to control in groves is non-synchronous flushes which prevents a one time spray from protecting all trees in a grove. It would be economically unfeasible to continue to spot spray as flushes occur within large acreages.

The primary control for young trees (less than 8 feet in height) should be soil-applied systemics such as imidacloprid. In IFAS trials, soil-applications of imidacloprid can provide up to 8 weeks control of pests including psyllids. Foliar sprays can also be used on young trees when soil-applications are not feasible or between soil-applications on a rotational program to minimize the potential for pesticide resistance.

6. How could existing psyllid biocontrol strategy fit into the management of HLB in Florida? What has been applied in foreign countries to control HLB spread? How effective is it? Address psyllid population fluctuations by season or related to host flushes, in residential, nurseries, and grove strategies. Are biocontrol agents we have in Florida efficacious at reducing psyllid populations and disease severity? (See the APHIS-Meyerdirk proposal).

This was discussed at length by the SP, (please see the report). Conclusions of the SP are that psyllid biocontrol is not likely to play a significant role in management of HLB. All instances that the SP was aware of where biocontrol has been utilized, they have been largely unsuccessful, with the exception of Reunion Island. The latter is really a special case, since it was a closed ecosystem.

Psyllid populations are known to fluctuate with the flushes of new citrus growth. This will differ with the species of citrus, the cultivar, and the age of the plant, as well as the cultural techniques (pruning, timing of fertilization) and environmental conditions. The best management tactic is to have monocultures of the same-aged cultivars planted or blocked together so that pesticide applications can be optimized (van den Berg. 1999. Neltropika Bulletin. 203:5-6). Nursery tactics should involve exclusion techniques such as screen houses, chemical treatments, and elimination of host material in surrounding areas. The best way to tackle the residential issue is through education and extension since backyard plantings include a wide assortment of varieties with different management styles. All indications are that one biocontrol agent has become established in Florida, and that it does appear in numbers late in the season. The key predators of the psyllid in FL are coccinellids, and their predation on parasitized nymphs may be a contributing factor to the ineffectiveness of the one introduced parasitoid (Michaud. 2004. Biological Control. 29:260-269). Still, biocontrol is not expected to have a significant effect on the spread of disease. Furthermore, biocontrol is not compatible with insecticide strategies used in commercial citrus production and these practices are not likely to change in the foreseeable future. However, chemical controls will be implemented primarily in groves, leaving wild rutaceous hosts and many backyard citrus trees and ornamentals unhindered to psyllid feeding and oviposition. This is where biocontrol may play a minor role in population suppression.

7. Could pheromone traps (if available) be employed to help eradicate / control psyllids in areas? Would it be possible to develop bait stations using spinosad coupled with a pheromone?

There is no known citrus psyllid pheromone and if one could be developed, it would take considerable time to develop it into a useful system. Mass trapping using pheromones has not been very effective on many other insect species.

First, it would be necessary to find a pheromone, and it is unknown whether that will be successful, as mentioned above. There is no known pheromone of the Asian citrus psyllid and studies are currently being considered. Several other members of the family Psyllidae are under investigation (not yet published) for attraction and repulsion to odors and sex pheromones. If an effective pheromone trap could be developed, then baited traps could be used to reduce numbers of vectors. However, it needs to be kept in mind that since this is a vector-borne pathogen, vector control using a baited trap will have only limited benefit on the spread of the disease.